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**RESEARCH ARTICLE**

# Downy mildew incidence and pathogen variability on pearl millet in Burkina Faso

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## Abstract

Downy mildew (DM) status is not well documented in Burkina Faso, however it is the first disease observed on pearl millet. A field survey was conducted in the major pearl millet growing areas in 2015 rainy season to assess the prevalence of DM on farm and to collect isolates for characterization. Thirty-four pearl millet fields in 34 villages were assessed. Nine isolates were collected and maintained on the susceptible line. All the fields surveyed were infested by DM with a disease incidence ranging from 4.3–69.0%. Differential reactions to the DM isolates were found among the genotypes used in the screening. The DM isolates were clustered in three virulence groups corresponding to three different pathotypes. The most virulent isolates (DM5, DM15 and DM14) of each pathotype group was selected for use in screening of pearl millet lines for the development of DM resistant line in Burkina Faso. To avoid rapid breakdown of the resistance of varieties, they must be resistant to at least two different pathotypes. Therefore, gen pyramiding strategy must be used. As the pathogen goes through sexual reproduction, some recombination occurs with the risk of appearance of new pathotypes after each cycle of reproduction. Therefore, a regular monitoring mechanism of the pathogen must be in place to control the disease.

**Key words**: Disease incidence, latent period, field survey, isolates, virulence

## Introduction

*Sclerospora graminicola* (Sacc.) Schroet is an obligate parasite that causes downy mildew (DM), a major disease of pearl millet (Sharma *et al.,* 2011; Prakash *et al.,* 2014). DM causes considerable yield losses and is particularly devastating on genetically uniform single-cross $F_1$ hybrid cultivars of pearl millet (Gowda *et al.,* 2006; Sharma *et al.,* 2008). *Sclerospora graminicola* reproduces both asexually, by producing sporangia and sexually, through oospores. The fungus has a rapid asexual reproduction cycle and it produces a huge number of spores in a short time (Idris and Ball, 1984). It is largely heterothallic and obligate sexual reproduction (required to produce resting spores that can survive in the soil from the end of one rainy season to the start of the next) contributes to the development of new recombinants in the pathogen population (Singh, 1995), increasing genotypic variability.

As a consequence of the evolution of pathogen population variants, new sources of virulence get established, leading to a breakdown of the effective resistance of genetically uniform host genotypes (Sharma *et al.,* 2011). Studies of *S. graminicola* conducted in India and several countries in Africa via the International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN), revealed differences in virulence among population of downy mildew pathogen populations (Gwary *et al.,* 2007; Wilson *et al.,* 2008). Pathogenic variability was identified between Sclerospora graminicola from West Africa and India, and among isolates of pathogen from Burkina Faso (Ball, 1983). Temporal changes in virulence of *Sclerospora graminicola* were also reported when the same variety was grown for 3 to 4 years in the same field (Thakur *et al.,* 2003). In Burkina Faso, farmers cultivate pearl millet each year in their fields without crop rotation or fallow due to land limitations. Furthermore, the national pearl millet improvement program is moving towards breeding of population cross hybrids, and ultimately single-cross hybrids, which were expected to be more vulnerable to DM than current landrace and improved open-pollinated cultivars. As a result, pearl millet yield losses due to downy mildew have not been quantified in Burkina Faso. Therefore, it is imperative to assess the distribution and incidence of DM and characterizes pathogen populations from the major pearl millet production areas to identify pathotypes that can be used to screen breeding materials. Previously, the distribution and incidence of DM has been assessed through field survey of pearl millet (Rao *et al.,* 2005; Sharma *et al.,* 2011). Sclerospora graminicola diversity has been characterized through host differential screening (Singh, 1995; Thakur *et al.,* 2011). This study aimed at assessing the on-farm incidence of pearl millet DM and characterizes the variability in pathogenicity and virulence of isolate of *S. graminicola* from the major pearl millet area in Burkina Faso to inform on the extent of the disease in Burkina Faso.

## Materials and methods

### Field survey and collection of isolates

A survey was conducted to assess the incidence of DM in 34 villages across the country, identified between latitude $11^{o}$ North; $5^{o}$ North and longitude $4^{o}$ E; $4^{o}$ W. The coordinates were marked on the map of Burkina Faso, using Google maps (Fig 1). The mapped points were then cleaned to keep only points that were situated along roadside, close to a village and not situated in a reserve. During the survey, a Global Positioning System (GPS) GARMIN device was used to record coordinates to confirm the position of the site on the map. One pearl millet field was sampled around the mapped points. In each field, 5 subplots of 4 rows, with 15 plants per row were sampled, 4 at the corners and one in the center of the field. In each subplot, the total number of plant and the number of diseased plants were counted. Percent downy mildew incidence (DMI) was calculated for each field as per the following formula-

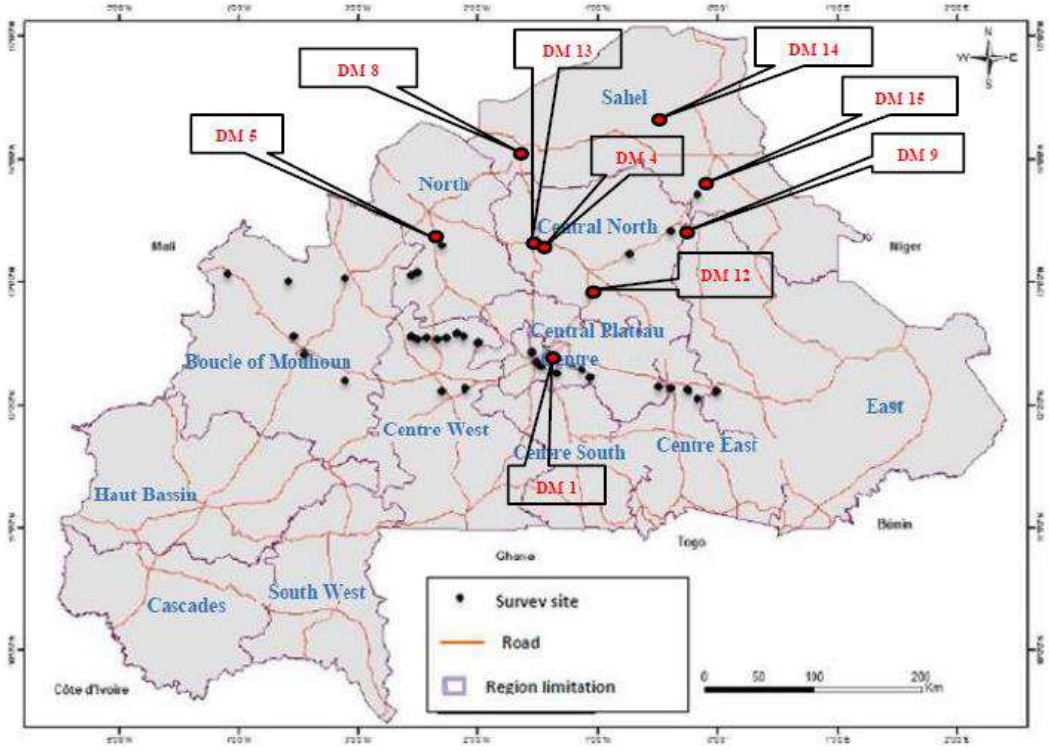
$$\text{DMI} = \frac{\text{Total number of diseased plants of field sublots}}{\text{Total number of plants of field subplots}} \times 100$$

Nine isolates of S. graminicola isolates were collected in farmers' fields by harvesting DM infected leaf samples on pearl millet. Isolates were collected in different regions of the country (Table 1, Fig. 1). The nine isolates were established from oospores on a highly susceptible line, 7042S, grown in pots, and maintained through asexual reproduction in isolation chambers in a greenhouse as described by Thakur *et al.,* (2011).

**Table 1: *Sclerospora graminicola* isolates found on pearl millet in farmers' field in the Sahelian and North-Sudanian zones of Burkina Faso**

| District of collection | Geographic coordinates | Identity of *S. graminicola* isolates |
|---|---|---|
| Saaba | 12.28436,-1.13056 | DM1 |
| Kongoussi | 13.32250,-1.53000 | DM4 |
| Ouahigouya | 13.29203,-2.29050 | DM5 |
| Djibo | 14.09690,-1.61780 | DM8 |
| Mani | 13.25810,-0.21310 | DM9 |
| Kaya | 13.09670,-1.08750 | DM12 |
| Kongoussi | 13.32250,-1.53000 | DM13 |
| Gorom | 14.44140,-0.23250 | DM14 |
| Dori | 14.04940,-0.02610 | DM15 |

**Fig 1: Map of Burkina Faso showing field survey sites including sites from which oospore samples were collected for establishment of pearl millet downy mildew isolates (DM1= downy mildew isolate number 1, DM4= downy mildew isolate number 4, DM5= downy mildew isolate number 5, DM8= downy mildew isolate number 8, DM 9= downy mildew number 9, DM12= downy mildew isolate number 12, DM13= downy mildew isolate number 13, DM14= downy mildew isolate number 14 and DM15= downy mildew isolate number 15)**




## Pathogenic diversity

### Host differential genotypes

A set of differential host lines obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), were used to characterized nine isolates of *S. graminicola* collected. The differential set consists of 14 genotypes identified by the International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN), one known susceptible line (7042S) and three additional genotypes from ICRISAT Niamey, Niger (Table 2).

**Table 2: Lines of pearl millet used in the study**

| Pedigree | Origin |
|---|---|
| 700651 (IP5082) | ICRISAT-IN |
| 852B | ICRISAT-IN |
| IP18292 (WSIL-P8) | ICRISAT-IN |
| IP18293 (IP18293-P152) | ICRISAT-IN |
| IP22291 (ICMB 841B-P3) | ICRISAT-IN |
| IP22295 (843B) | ICRISAT-IN |
| IP22313 (ICMB 88004) | ICRISAT-IN |
| IP22315 (ICMB 88006) | ICRISAT-IN |
| IP22319 (ICMB 90111-P2) | ICRISAT-IN |
| IP22439 (81B-P6) | ICRISAT-IN |
| IP22441 (7042-DMR) | ICRISAT-IN |
| IP22442 (ICMP 451-P6) | ICRISAT-IN |
| IP22445 (ICMP 85410-P7) | ICRISAT-IN |
| IP22446 (ICMR 356) | ICRISAT-IN |
| 38B | ICRISAT-SC |
| 51-4B | ICRISAT-SC |
| 7042-DMS | ICRISAT-SC |
| PE08407 | ICRISAT-SC |

**IN = India, SC = Sahelian Center**

**Inoculum preparation and inoculation**

Infected leaf samples from plants inoculated with each isolate were incubated at 20° C for 8 hours to allow production of sporangia. The sporulating leaf of each isolate were washed in ice-cold sterilized distilled water (4° C) to harvest sporangia. Spore concentration was adjusted to 2.4 × 105 mL-1. The inoculum of each isolate was used to spray-inoculate seedlings (two-leaf stage) of the 18 differential cultivars. Inoculated seedling were incubated in the dark for 16 hours at 20° C and relative humidity >90%. The experiment, with individual isolates was conducted in a randomized complete block design (RCBD) with three replications and 25–30 seedlings per differential line per replication.

**Data collection and analysis**

The characterization of symptom development for each isolate of *S. graminicola* was done following the method proposed by Thakur and Rao (1997). Latent period, number of days from inoculation to appearance of the first typical symptom of downy mildew on seedlings, was recorded from the second day post inoculation. The Incidence of downy mildew (DMI), the quantitative measure of the disease-causing potential of isolates were calculated as percent of infected seedlings 14 days after inoculation. The virulence index was calculated as the disease incidence × latent period-1 to determine quantitative differences in virulence among isolates. Data were submitted to analysis of variance (ANOVA) to determine significant difference among the isolates of *S. graminicola.* Cluster analysis was performed using the average linkage method to identify group of pathogens. Analyses were performed using GenStat statistical package edition 2012 (Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK).

## Results and discussions

### Prevalence and extent of downy mildew in farmers' fields

During this study, downy mildew was found in all the thirty-four fields surveyed with different level of disease incidence ranging from 4.3 to 69.0% (Table 3). Of the 34 fields, only three showed low disease pressure (<10.0% disease incidence), located in the villages of Nagrego in the Central region, Djoubasso in the north-western region and Moada in the Eastern region. The highest disease incidence (69.0%) was recorded at Tosson in the north-west of the country. In general, the highest disease incidences (>50%) were frequently recorded in the field in the North-Sudanian zone (Bonou, Arbole and Tosson). However, there was no specific region with consistently low or high disease pressure. This is the first study in Burkina Faso of assessing the incidence of downy mildew in farmers' field through field survey. The presence of DM symptoms in all the pearl millet fields surveyed clearly indicate that downy is widespread in pearl millet production areas in Burkina Faso. It constitutes an important yield limiting factor as high disease incidences were observed in many of the fields surveyed. In fact, pearl millet yield losses in Africa due to DM, was estimated to 10-60% (Nene and Singh, 1976 and Shetty *et al.*, 2016). There is no report on the assessment of direct yield loss due to DM in Burkina Faso. However, it is reported that yield loss due to DM is proportional to the disease incidence (high positive correlation) (Williams and Singh, 1981). The results of this study are much closer to a previous five-year study on DM field screening in Burkina Faso where DM incidence varied from 8 to 57% with 72% on the susceptible line (Thakur *et al.*, 2004). The current field survey result also confirms farmer perceptions that DM is an important disease of pearl millet in Burkina Faso (Drabo *et al.*, 2018). Even though farmers are growing only open-pollinated varieties (OPVs) up to date, and genetically heterogeneous OPVs are known to be moderately resistant to DM compared to genetically uniform single-cross $F_1$ hybrids, the continuous cultivation of similar genetic materials in the same field is reported to contribute to increase the susceptibility of farmers' landraces to DM. Furthermore, due to sexual reproduction, new virulence combinations continuously arise in the pathogen population, and some new pathogens may be capable of overcoming the resistance of the cultivar.

### Downy mildew pathogen variability in Burkina Faso

There were significant differences ($P < 0.01$) among the isolates for the latent period, the disease incidence, and the virulence index as response of the differential cultivars to the different *S. graminicola* isolates (Table 4). Of the eighteen host genotypes, 12 showed differential reaction (Table 5). Incidence of DM varied from 29.0 to 100%. The mean disease incidences across the 18 host genotypes ranged from 68.0% (DM9) to 97.0% (DM5) (Table 5).

The cluster analysis using the virulence index data revealed three virulence groups (Fig. 2). Group I, comprising of three isolates (DM1, DM13 and DM5) was the most virulent group, with a mean virulence index >14; followed by Group III comprising one isolate (DM14) with a mean virulence index of 12.3. Group II, comprising 5 isolates was the least virulent group with a mean virulence index <12. The genotypes screened were clustered in two groups (G1 and G2) based on their reaction to the nine isolates of *S. graminicola* isolates (Fig 3). Cluster 2 (G2) was composed of seven differential lines and was more efficient in characterizing the isolates as it clusters the most virulent isolates of the different pathotype groups separately.

**Table 3: Downy mildew incidence (DMI) on pearl millet landraces in farmer's fields in villages across the Sahel and the North-Sudan zones of Burkina Faso**

| Village | DMI | No | Villages | DMI |
|---|---|---|---|---|
| Nagrego | 4.3 | 18 | La-Toden | 19.7 |
| Djoubasso | 4.7 | 19 | Konankoira | 20.3 |
| Moada | 5.7 | 20 | Nakamtenga | 22.0 |
| Ouahigouya | 11.0 | 21 | Koupela | 27.3 |
| Kougri | 11.7 | 22 | Wend-Nong-Tenga | 32.3 |
| Bomborokouy | 11.7 | 23 | Nabrabaogo | 37.0 |
| Katchari | 12.0 | 24 | Bassan | 37.3 |
| Mogtedo | 12.7 | 25 | Tikan | 37.7 |
| Fada | 13.0 | 26 | Yaba | 40.0 |
| Songnaba | 13.3 | 27 | Laye | 42.3 |
| Kansara | 15.0 | 28 | Kaya | 43.3 |
| Ralo | 16.3 | 29 | Guimou | 44.0 |
| Tanghin | 16.3 | 30 | Bani | 47.7 |
| Sassa | 16.7 | 31 | Napalgue | 49.0 |
| Zorgho | 16.7 | 32 | Bonou | 54.0 |
| Koulkouldi | 17.0 | 33 | Arbole | 56.0 |
| Korsomoro | 17.7 | 34 | Tosson | 69.0 |
| Mean | | | | 26.3 |
| SE ± | | | | 9.02 |
| P value | | | | <0.001 |
| CV (%) | | | | 34.3 |

**Table 4: Means squares for latent period, downy mildew incidence and virulence index for nine isolates of *Sclerospora graminicola* collected from pearl millet in Burkina Faso**

| Source of variation | DF | Latent period | DM incidence | Virulence index |
|---|---|---|---|---|
| Replication | 2 | 0.5711 | 0.961 | 5.456 |
| *S. graminicola* isolates | 8 | 42.5502** | 747.051** | 301.467** |
| Host genotypes | 17 | 49.8781** | 24.276** | 497.065** |
| Isolate × Host genotypes | 136 | 2.2302** | 3.049** | 13.693** |
| Residual | 306 | 0.9764 | 1.059 | 4.71 |

** = significant at $P < 0.01$,

The differential reaction of the host genotypes towards the *S. graminicola* isolates (Table 5) shows pathogenic variation in the pathogen populations. Previous work has demonstrated variation in host-genotype resistance, occurrence of host-pathogen specificity in pearl millet DM (Thakur and Rao, 1997; Vanderplank, 2012). Variation in the pathogen population for virulence in the host genotypes is necessary for selection of host-specific virulence (Thakur *et al.*, 1992). In pearl millet DM pathosystem, the level of disease incidence is an indication of quantitative differences in virulence in the pathogen and resistance in the host genotype.

**Table 5: Downy mildew incidence on eighteen pearl millet host differential cultivars inoculated individually with nine isolates of *S. graminicola* in farmer fields in Burkina Faso**

| Differential host lines | Isolate and mean downy mildew incidence (%) | | | | | | | | | Trial mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | DM1 | DM12 | DM13 | DM14 | DM15 | DM4 | DM5 | DM8 | DM9 | |
| 700651 | 95 | 64 | 95 | 34 | 77 | 73 | 87 | 94 | 34 | 73 |
| 852B | 99 | 76 | 96 | 68 | 81 | 92 | 100 | 71 | 41 | 80 |
| IP18292 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| IP18293 | 89 | 62 | 93 | 90 | 100 | 43 | 93 | 77 | 49 | 77 |
| IP22291 | 91 | 90 | 99 | 72 | 100 | 94 | 96 | 99 | 58 | 89 |
| 843B | 99 | 100 | 100 | 100 | 100 | 100 | 99 | 100 | 100 | 100 |
| IP22313 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| IP22315 | 91 | 83 | 83 | 81 | 83 | 80 | 91 | 64 | 69 | 81 |
| IP22319 | 100 | 99 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 81B-P6 | 96 | 92 | 92 | 98 | 77 | 94 | 98 | 86 | 51 | 87 |
| 7042-DMR | 85 | 76 | 71 | 85 | 98 | 92 | 94 | 58 | 56 | 79 |
| IP22442 | 100 | 100 | 100 | 67 | 100 | 99 | 100 | 94 | 97 | 95 |
| IP22445 | 100 | 86 | 89 | 95 | 78 | 85 | 98 | 71 | 54 | 84 |
| IP22446 | 93 | 83 | 98 | 94 | 99 | 100 | 100 | 98 | 75 | 93 |
| 38B | 67 | 95 | 82 | 37 | 86 | 44 | 90 | 76 | 33 | 71 |
| 51-4B | 97 | 75 | 77 | 35 | 83 | 91 | 98 | 66 | 29 | 72 |
| 7042-DMS | 100 | 100 | 100 | 99 | 100 | 100 | 100 | 100 | 88 | 99 |
| PE08407 | 100 | 99 | 100 | 100 | 100 | 100 | 100 | 98 | 91 | 99 |
| Mean | 95 | 87 | 93 | 80 | 92 | 88 | 97 | 85 | 68 | 87 |
| SED | 2.413 | | | | | | | | | |

**Fig 2: Cluster analysis classification of nine isolates of *S. graminicola* collected from pearl millet from different fields in Burkina Faso based on the virulence index data from 18 host differential inbred lines**

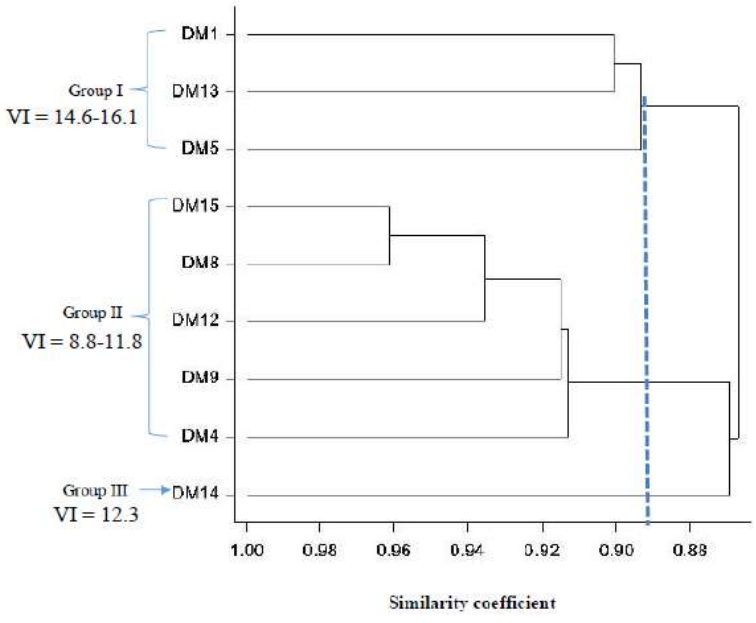
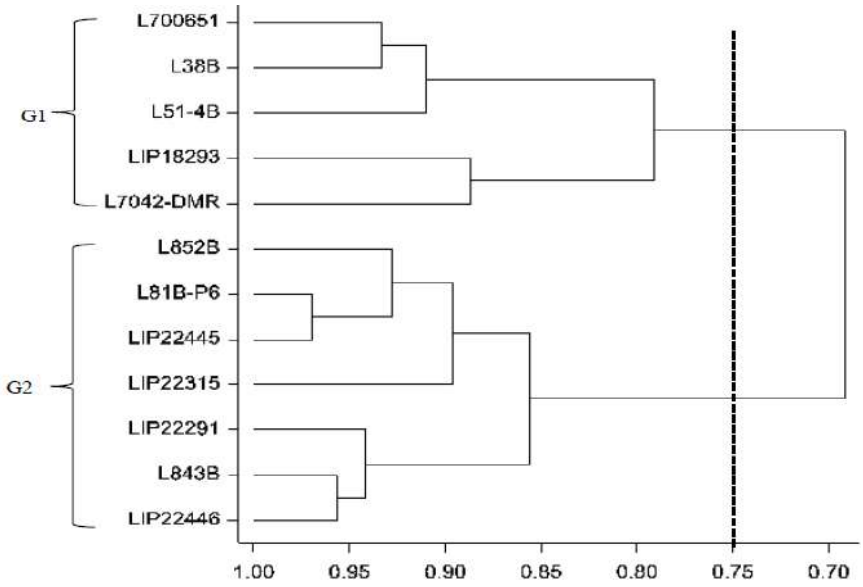


**Fig 3: Cluster analysis classification of 12 differential lines of pearl millet based on reaction to nine isolates of *S. graminicola* collected from pearl millet from different fields in Burkina Faso**




In this study, the DM incidence was high, indicating the high virulence of downy mildew in farmers' fields, but the virulence was variable in 12 out of the 18 host genotypes used in the screening. The genotypes from ICRISAT Niger originated from West Africa were more resistant compared to the genotypes from India; indicating that differential lines derived from Africa-based breeding programs (700651, 38B and 51-4B), are more resistant than those bred in India, and this would support greater investment in breeding for pearl millet DM resistance in Africa, particularly if breeding programs are working toward hybrid cultivars to improve grain yield potential and reduce vulnerability to DM. The high disease incidence (88 – 100%) recorded on the susceptible check (7042-DMS) was an indication of a high disease pressure in this screening. Based on the virulence index, the nine isolates were grouped in to three pathotypes. The existence of three pathotypes in the nine isolates further supports the presence of a range in virulence in the DM pathogen in Burkina Faso.

## Conclusion

This study was a systemic survey in farmer fields across Burkina Faso. The results obtained revealed the extend of downy mildew in the country. Downy mildew occurred on landraces in the entire field surveyed with different importance, ranging from low to high. The evidence of the existence of diverse pathotypes was also proven. Three virulence groups were identified. The findings from this study identified DM as the most important disease of pearl millet and a priority trait in pearl millet breeding in Burkina Faso. It could be concluded also that DM pathotypes from Burkina Faso seem to be more virulent than Indian pathotypes. There is a need to develop a new set of differential lines in West Africa. The three virulence groups must be maintained and used to systematically screen all breeding lines. A regular monitoring of the downy mildew on farm would be useful in identifying new virulent.

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